DEVELOPMENT OF HALF-EGGS OF ARBACIA PUNCTULATA OBTAINED BY CENTRIFUGING AFTER FERTILIZATION, WITH SPECIAL REFERENCE TO PARTHENOGENETIC MEROGONY

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It has been shown previously (1932, 1936) that the half-eggs of *Arbacia punctulata*, which are obtained by centrifuging the *unfertilized* egg, will develop if fertilized or activated artificially. The white halves, containing the ♀ nucleus will develop often quite normally, both fertilized and parthenogenetic, into plutei; the red halves after fertilization (fertilized merogones) containing only the ♂ nucleus will develop, usually in an aberrant fashion, into plutei. The red halves will also develop parthenogenetically (parthenogenetic merogones) as far as the blastula stage, in spite of the fact that they have no nuclei whatever. Additional data with a more complete series of photographs on this subject will be published in a forthcoming number of this journal.

The present paper deals with the development of similar half-eggs of *Arbacia punctulata* which are obtained by centrifuging the *fertilized* egg. It might be expected that these halves would develop similarly to (or better than) those mentioned above, having the same nuclear (or non-nuclear) content. Such has been found, however, not to be the case; they do not develop nearly so well.

Stratification and Breaking of the Fertilized Egg

When *Arbacia* eggs which have been normally fertilized are centrifuged, they stratify into layers similar to the unfertilized egg, only the layers are not nearly so clean-cut (Photograph 1). Except for about five minutes after fertilization, the fertilization membrane prevents the elongation and breaking apart of the egg, so that it is necessary to remove this in order to obtain the half-eggs. This is removed by shaking the eggs 2 minutes after fertilization, just after the membrane has been raised and before it has hardened. The eggs were centrifuged for 6 to 8 minutes at about 10,000 × g., in an isosmotic sugar solution (approximately 2 parts sugar solution to one part sea water + eggs). At one stage of very short duration soon after fertilization, the egg tends

to break up into very small pieces when centrifuged, as noted previously (1933); at all other times, it breaks into slightly unequal halves, similar to the unfertilized egg (Photograph 2). At most stages, the white half is slightly larger than the red, but if centrifuged just before cleavage, this half tends to be considerably smaller (Photograph 3). The long streamers which have been described as characteristic of the fertilized egg at a certain stage and which are readily observed with the centrifuge microscope (1933), are preliminary to the final breaking apart and are especially noticeable with relatively low centrifugal forces (5,000 × g.). When the streamers break apart, a sort of tail is often left on the white half; this contracts after 10 to 30 minutes, leaving the white half-egg spherical. The nucleus, which soon after fertilization is the combined ♂ and ♀ nuclei, always goes to the light pole, and is therefore always in the white half, just as is the female nucleus in the unfertilized white half. The ♂ nucleus in the recently fertilized *Arbacia* egg is apparently lighter than the pigment and yolk granules and is not carried by centrifugal force into the heavy half-egg, as it is in the eggs of *Parechinus microtuberculatus, Paracentrotus lividus* and *Sphaerechinus granularis* (1934). It is only in those eggs in which the sperm has entered near the centrifugal end of the egg, as it is thrown into position in the centrifuge, and the egg centrifuged apart while the sperm nucleus is still in this position, that any nucleus is found in the red half-egg which has been completely separated off. This will be described later on. If centrifuged after the amphiaster has been formed, this also, together with the chromosomes, goes to the light pole and is segregated in the white half-egg.

Development of the White Half-egg

The early development of the white half may be fairly normal. The first cleavage divides the egg into two equal cells, through the oil cap (Photograph 4), or through the plane of stratification (Photograph 5) or diagonally; there is a delay of only a few minutes in comparison with the controls. Normal 4 and 8-cell stages follow (Photographs 6, 7), and with further cleavages (Photographs 8–10) a blastula is formed slightly later than the normally developing egg. Owing to the lack of pigment and the small size of the cells, it is difficult to be sure about the micromeres; in a few cases I have thought they were present, but usually they could not be observed. Many of the blastulae, after becoming free-swimming, develop no further though they increase slightly in size—they become "Dauerblastulae," or permanent blastulae (Photograph 11). Some of the blastulae become filled with cells and somewhat differentiated (Photograph 12), some acquire a skeleton often

in the form of a primitive triradiate spicule, sometimes more complicated, even without invagination; that is, the formation of the skeleton seems independent of the shape of the larva or other differentiation (Photograph 13). None of the blastulae has developed into a normal pluteus. The nearest approach to a pluteus is shown in Photograph 14; the skeleton approximates the normal, but there are no arms and it is only about a fifth the volume of the normal white pluteus of this age (4 days) from a white half obtained before fertilization and subsequently fertilized. Similar permanent blastulae and imperfect plutei occur together with normal plutei in the white halves fertilized after centrifugation (Photographs in succeeding paper).

Many of the white halves do not develop so normally as this. It has been noted in previous papers (1934, 1940) that the ectoplasmic layer which is formed soon after fertilization and binds the cleavage cells together is thrown off by centrifugal force. In some cases, in the

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PLATE I

Development of White Half

The photographs were all taken of living eggs and all brought to approximately the same magnification, 250 ×.

PHOTOGRAPH 1. Fertilized egg centrifuged 4 minutes after fertilization. Photographed immediately; to show stratification.

PHOTOGRAPH 2. Typical red and white halves. Centrifuged 5 minutes after fertilization. Photographed 10 minutes later. Note nucleate white halves, with contracted tail, and non-nucleate reds.

PHOTOGRAPH 3. White half much smaller than red. Centrifuged just before cleavage. Photographed one-half hour later.

PHOTOGRAPH 4. Two-cell stage, cleavage through the oil cap. Centrifuged 11 minutes after fertilization. Photographed 1 hour after fertilization.

PHOTOGRAPH 5. Two-cell stage, cleavage parallel with stratification. Centrifuged 25 minutes after fertilization, streak stage. Photographed 1 hour after fertilization.

PHOTOGRAPH 6. Four-cell stage. Centrifuged 6 minutes after fertilization, monaster stage. Photographed 1¼ hours after fertilization.

PHOTOGRAPH 7. Eight-cell stage. Centrifuged a little before cleavage. Photographed 2½ hours after fertilization.

PHOTOGRAPH 8. Eight to 16-cell stage. Centrifuged 30 minutes after fertilization, streak stage. Photographed 2½ hours after fertilization. Note red halves uncleaved.

PHOTOGRAPH 9. Early blastula. Centrifuged a little before cleavage. Photographed 7 hours after fertilization. Note red half uncleaved.

PHOTOGRAPH 10. White ciliated blastula, 1 day old. Centrifuged 40 minutes after fertilization.

PHOTOGRAPH 11. White ciliate blastula, "Dauerblastula," 2 days old. Centrifuged 15 minutes after fertilization, early streak stage.

PHOTOGRAPH 12. White blastula, 4 days old, somewhat differentiated. Same lot as above.

PHOTOGRAPH 13. Four-day white blastula with triradiate spicule. Same lot.

PHOTOGRAPH 14. Abnormal pluteus, 4 days old, same lot. Most normal pluteus found.

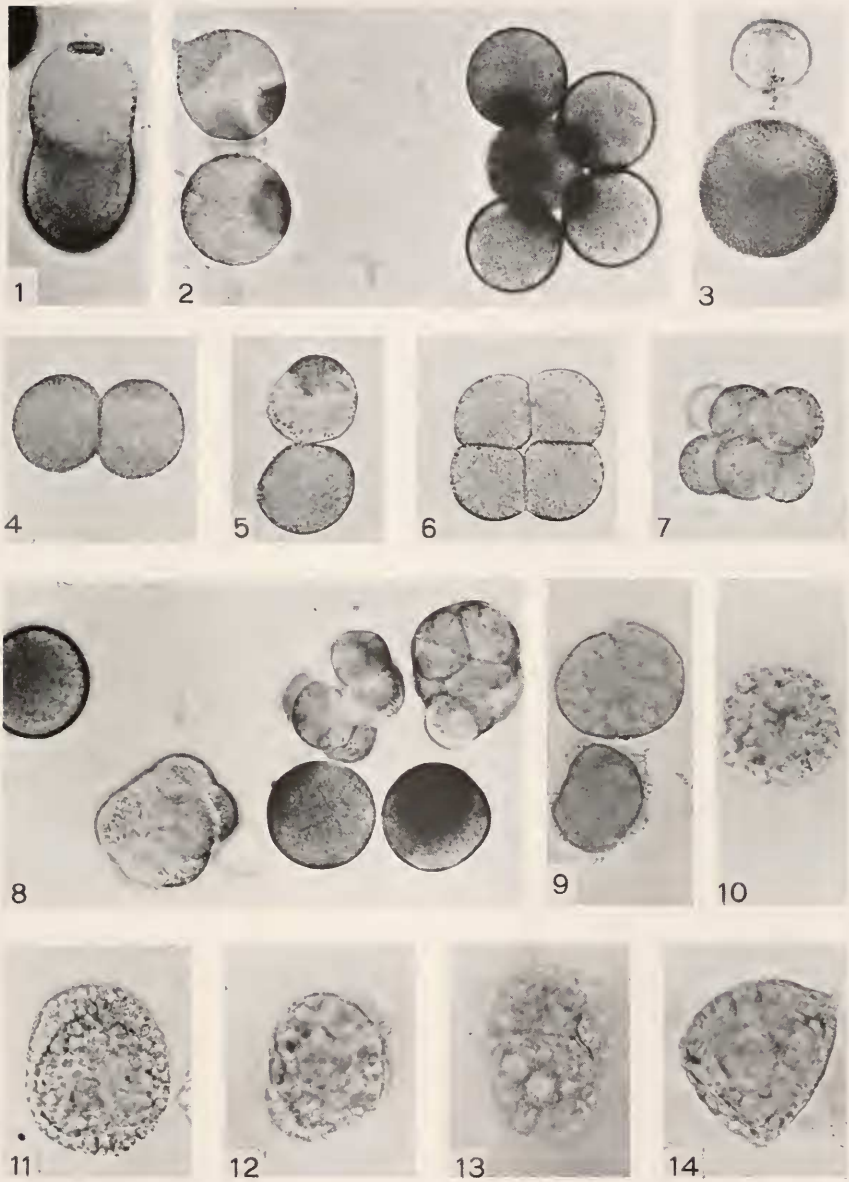

PLATE I

white halves obtained after fertilization, this layer does not regenerate sufficiently to hold the first two blastomeres together (Photograph 15). Each blastomere develops quite independently, each cleaving in regular sequence to form a white blastula (Photographs 16–21). A pair of white twins is formed which swim at first in pairs quite similar to (but of course smaller than) the twins of the whole egg obtained by centrifuging the fertilized egg at the two-cell stage (1940). These white twins gastrulated, but those isolated had formed no skeleton after 3 days.

Together with these two modes of development of the white halves a less normal development may take place. The white half after having been broken off by centrifugal force, often becomes amoeboid, or it may become amoeboid after several cleavages (Photographs 22–24). It also frequently forms a number of loosely united cells which by further cleavages give rise to a large mass of unorganized cells (Photographs 25, 26). The lack of the ectoplasmic layer is no doubt responsible for this scattering of cells.

Red Half-egg

The red half-egg contains no nucleus but consists of protoplasm which has previously been normally fertilized. These halves often do

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PLATE II

Development of White Half, Continued

PHOTOGRAPH 15. First two white blastomeres remain apart. Centrifuged just before cleavage. Photographed 1½ hours after fertilization. Note red half uncleaved.

PHOTOGRAPH 16. Each white blastomere has cleaved independently. Centrifuged 23 minutes after fertilization. Photographed 1¾ hours after fertilization.

PHOTOGRAPH 17. Each white blastomere is 4-celled. Centrifuged 6 minutes after fertilization. Photographed 2 hours after fertilization.

PHOTOGRAPH 18. Late cleavage of white twins. Centrifuged 40 minutes after fertilization. Photographed 4 hours after fertilization. Note whole blastula below.

PHOTOGRAPH 19. White twin blastulae after becoming free-swimming. Centrifuged 23 minutes after fertilization. Photographed 7 hours after fertilization.

PHOTOGRAPH 20. White twin blastulae, with undeveloped red half. Centrifuged a little before cleavage. Photographed 7½ hours after fertilization.

PHOTOGRAPH 21. A similar pair with whole blastula in comparison. Centrifuged 40 minutes after fertilization. Photographed 7½ hours after fertilization.

PHOTOGRAPH 22. White half amoeboid. Centrifuged 23 minutes after fertilization. Photographed 1½ hours after fertilization.

PHOTOGRAPH 23. White half very amoeboid. Centrifuged 42 minutes after fertilization. Photographed 1½ hours after fertilization.

PHOTOGRAPH 24. White half amoeboid after several cleavages. Centrifuged just before cleavage. Photographed 3 hours after fertilization. Red half undeveloped.

PHOTOGRAPH 25. White halves form scattered cells. Centrifuged 30 minutes after fertilization. Photographed 2½ hours after fertilization.

PHOTOGRAPH 26. Same group as one shown in Photograph 25, after further cleavages, 1 hour later.

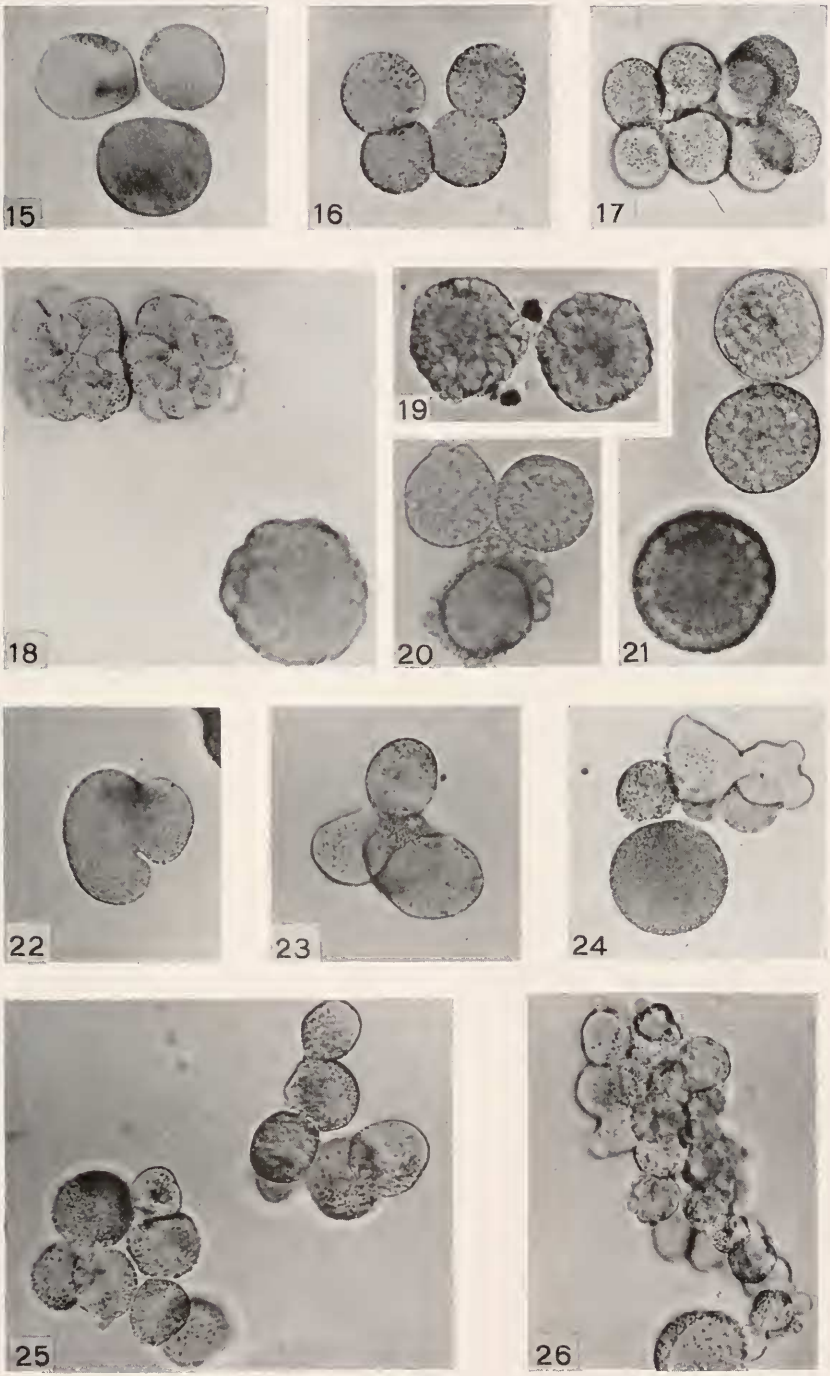

PLATE II

not change at all even after several hours and do not develop (Photograph 27). In many cases, however, they show signs of activation in that they become amoeboid (Photographs 28, 29). Often a notch appears in the middle of the egg, as though it were about to cleave (Photographs 30, 31). A series of photographs (Photographs 33–36), at intervals of about 10 minutes, indicates that the cleavage does not actually take place, but the notch seems to be an abortive attempt to cleave and soon disappears. The red half sometimes breaks up into a number of small spheres (Photograph 32), but this seems to be by a pinching-off process rather than cleavage as there are no preliminary stages except the amoeboid processes noted above, and the spheres are unstable, that is, they come and go, and they do not become progressively smaller. That the protoplasm is activated and different from unfertilized protoplasm is indicated also by the occasional appearance of a large monaster (Photographs 37, 38), and in one red half a beautiful amphiaster was observed, but this did not lead to cleavage (Photograph 39).

It makes very little difference at what stage after fertilization the eggs are broken apart by centrifugal force. It seemed to me reasonable to suppose that after the nuclear membrane had broken and liberated its contents into the cytoplasm, then this cytoplasm would be different, and the red half would be more likely to cleave than previously. This was, however, not the case. The red halves obtained after the breakdown of the nuclear membrane act exactly like those obtained while the nuclear membrane is still intact; there is no cleavage. This means either that all the achromatic material from the nucleus goes together with the chromosomes and spindle into the light half, or else that it has no effect on the protoplasm, in enabling it to cleave.

PLATE III

Red Halves

PHOTOGRAPH 27. Red halves unchanged after 5 hours. Centrifuged 15 minutes after fertilization.

PHOTOGRAPHS 28, 29. Red halves amoeboid. Centrifuged 30 minutes after fertilization, streak stage. Photographed 4 hours after fertilization.

PHOTOGRAPH 30. Red halves with notch as though ready to cleave. Centrifuged a little before cleavage. Photographed 2½ hours after fertilization.

PHOTOGRAPH 31. Red halves notched and amoeboid. Centrifuged 30 minutes after fertilization. Photographed 3 hours after fertilization.

PHOTOGRAPH 32. Red halves pinched into small fragments. Centrifuged 10 minutes after fertilization, monaster stage. Photographed 5 hours later.

PHOTOGRAPHS 33–36. One red half at intervals of 10 minutes. Centrifuged a little before cleavage. Photographed 1½–2 hours after fertilization.

PHOTOGRAPHS 37, 38. Red halves with monaster. Centrifuged 6 minutes after fertilization. Photographed 3 hours later.

PHOTOGRAPH 39. Red half with amphiaster. Centrifuged 21 minutes after fertilization. Photographed 2 hours later.

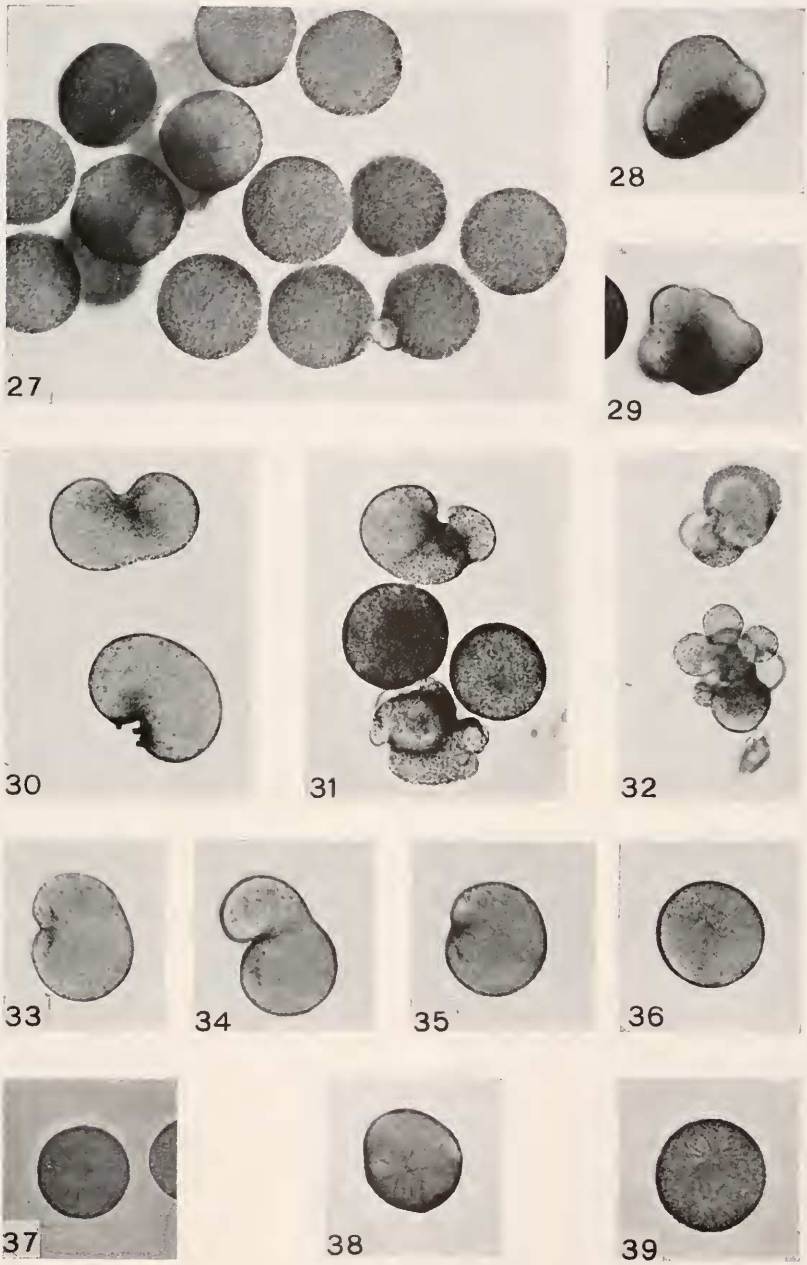

PLATE III

Re-activation of Halves

The red halves obtained by centrifuging the fertilized egg cannot be re-fertilized nor can they be artificially activated. Even if obtained as quickly as possible after fertilization, re-activation has practically no effect. This is true also of the white halves; there was only a slightly greater tendency toward amoeboid activity and disorganized masses of cells if treated with the hypertonic solution which causes parthenogenesis in the unfertilized halves.

Development of Halves Obtained Immediately After Fertilization

As mentioned above, it is only if one centrifuges the eggs immediately after fertilization, that one can obtain any cleavage in the red halves. This cleavage is due to the presence of the sperm which entered near the centrifugal pole, and the egg has broken apart before the sperm nucleus has had a chance to approach the ♀ nucleus at the centripetal pole. It is well known that the sperm may enter the *Arbacia* egg at any point on the surface, and also that the eggs fall at random in the centrifuge tubes without any orientation. A group of red halves from eggs centrifuged immediately after fertilization is shown in Photograph 40. It is seen in the next photograph (41) that one of these halves contains the ♂ nucleus, and in the two succeeding photographs (42, 43) it is shown that this half-egg cleaves whereas the others do not. If the red half contains the ♂ nucleus, it develops similarly to a

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PLATE IV

Development of Halves Obtained Immediately After Fertilization

PHOTOGRAPH 40. Group of red halves centrifuged off 3 minutes after fertilization. Photographed 1 hour later; monaster in center cell.

PHOTOGRAPH 41. Same group ½ hour later; ♂ nucleus in center cell.

PHOTOGRAPH 42. Same group ½ hour after 41; center cell cleaving. Monaster in egg above to left.

PHOTOGRAPH 43. Same group 20 minutes after 42; center cell completely cleaved, with nucleus in each cell. ♂ nucleus in egg above.

PHOTOGRAPH 44. Development of red half with ♂ nucleus; 3-celled, multinucleate. Same set as above. Photographed 6 hours after fertilization.

PHOTOGRAPH 45. Development of red half, multinucleate. Centrifuged 1 minute after fertilization. Photographed 7 hours later.

PHOTOGRAPH 46. Group of small pigmented fragments, one with ♂ nucleus. Centrifuged 1 minute after fertilization. Photographed 1 hour later.

PHOTOGRAPH 47. Same lot, another fragment 4 hours later; now 2-celled.

PHOTOGRAPH 48. White half centrifuged 4 minutes after fertilization. Photographed 1½ hours later; normal development.

PHOTOGRAPH 49. White half centrifuged 3 minutes after fertilization. Photographed 3 hours later; normal development.

PHOTOGRAPH 50. White half centrifuged 4 minutes after fertilization. Photographed 3 hours later; disconnected cells.

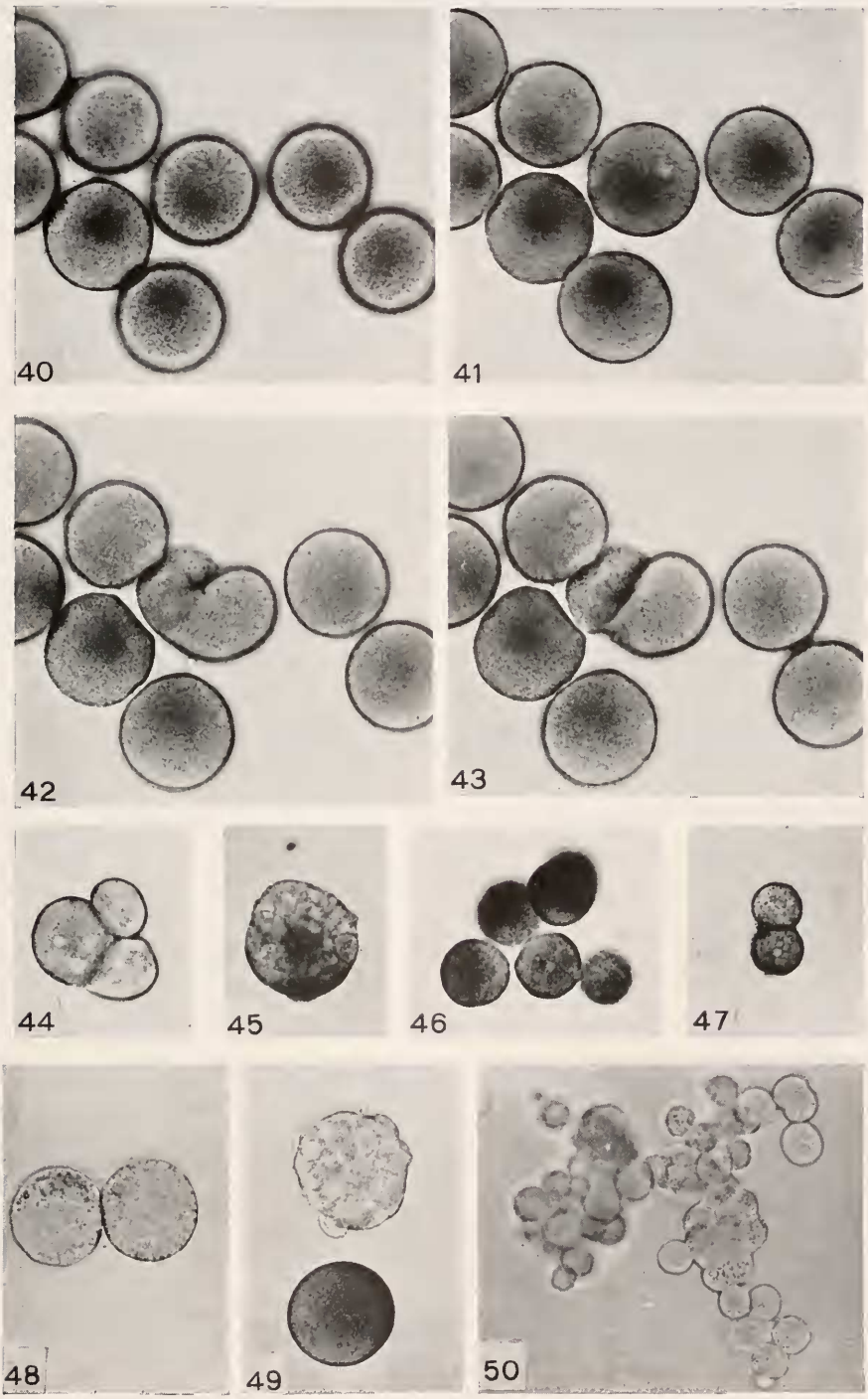

PLATE IV

red half fertilized after being separated from the white half (i.e. a fertilized merogone), nuclear divisions often taking place without cell divisions (Photographs 44, 45). If centrifuged at a certain period soon after fertilization when the eggs tend to break into small pieces, it occasionally happens that the sperm is entrapped in one of the small heavy fragments (Photograph 46, center cell). This, though quite small, will cleave (Photograph 47); similar fragments without the male nucleus do not cleave.

The white halves obtained by centrifuging the eggs immediately after fertilization, and usually containing both nuclei [1] develop similarly to those broken off at later periods, sometimes cleaving quite regularly (Photographs 48, 49), and sometimes forming irregular masses of disconnected cells (Photograph 50).

Comparison with Parthenogenetic Merogones, and Discussion

The lack of development of the red halves from the fertilized egg is in sharp contrast to the development of the red halves obtained from the unfertilized egg and subsequently activated artificially, the parthenogenetic merogones. These, as I have shown (1936), will cleave in a fairly orderly fashion until they become blastulae. Photographs 51–54 show some successive stages in the development of a group of the parthenogenetic merogones, the unfertilized red halves (51), a group soon after activation (52), and two successive stages in cleavage (53, 54). A few more photographs of fairly normal cleavages are also

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PLATE V

Parthenogenetic Merogones

PHOTOGRAPH 51. Group of red halves obtained by centrifuging the unfertilized egg.

PHOTOGRAPH 52. Similar red halves 3 hours after activation with hypertonic sea water. Note fertilization membrane and ectoplasmic layer, and monaster in lower right egg.

PHOTOGRAPH 53. Similar red halves 4 hours after activation. Early cleavage.

PHOTOGRAPH 54. Same lot of eggs ½ hour later. Further cleavages.

PHOTOGRAPH 55. Four-cell parthenogenetic merogone, 4 hours after activation.

PHOTOGRAPH 56. Eight-cell stage, 4½ hours after activation.

PHOTOGRAPH 57. About 16-cell stage, 7 hours after activation.

PHOTOGRAPH 58. Early blastula of parthenogenetic merogone, 27 hours after activation.

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[1] For development of these white halves as watched with the centrifuge microscope see 1933, p. 394; it is difficult to tell whether they have both ♂ and ♀ nuclei or only the ♀ in mass cultures.

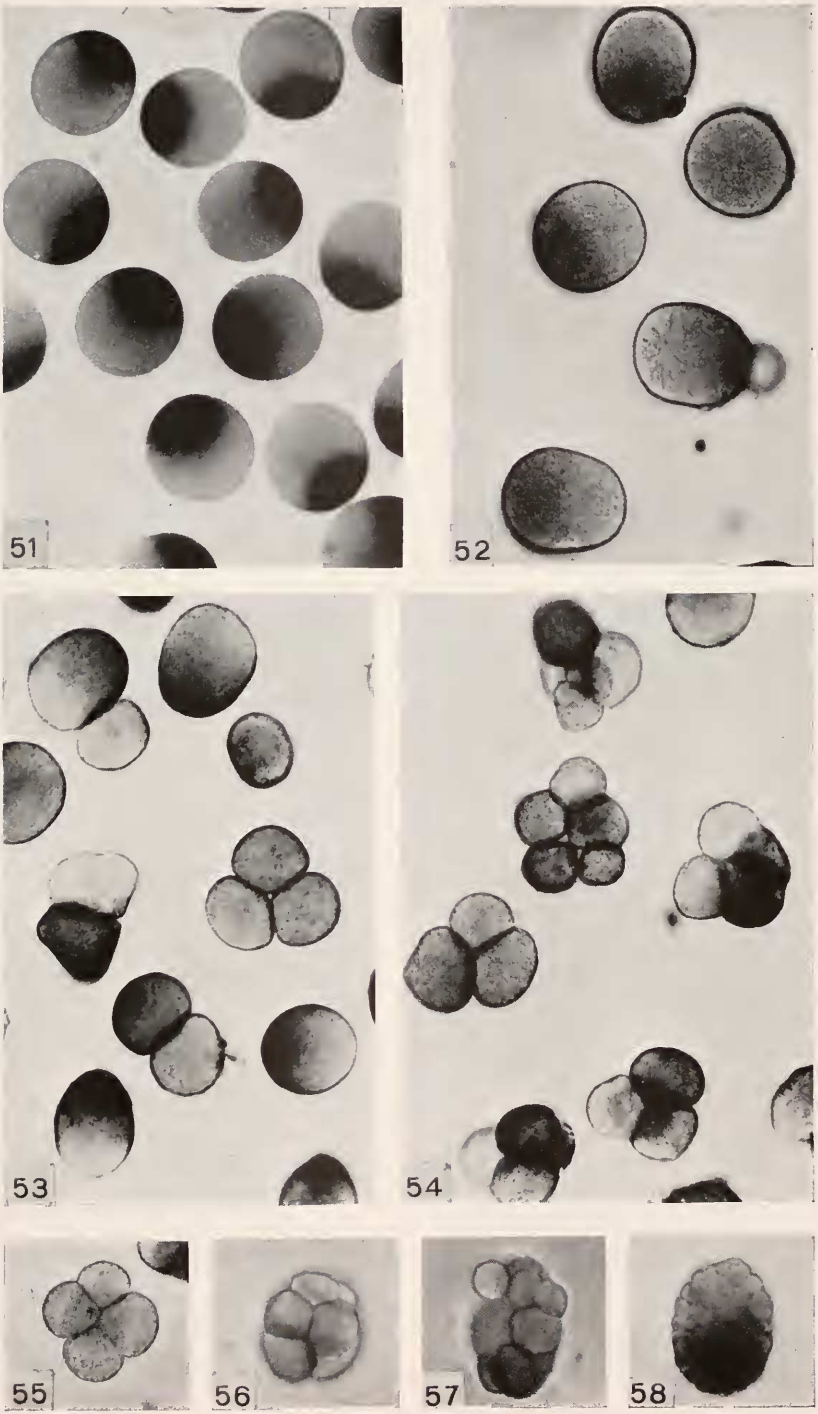

PLATE V

presented (Photographs 55–58). I wish especially to call attention to the very normal early blastula (Photograph 58).

It seems extraordinary that these red halves which have been activated artificially, without ever having had any contact with sperm or substances from the mature female nucleus, should develop better than the red halves, obtained after fertilization, which consist of protoplasm which has been acted upon by sperm in a normal manner, and which in some cases (when centrifuged after the breakdown of the nuclear membrane) has been mixed with the substances of the nucleus. It might be emphasized here that the fact that the germinal vesicle has liberated substances into the cytoplasm before the occurrence of parthenogenetic merogony has not escaped my notice, and this was particularly discussed in my first paper (1936, p. 119). This in itself cannot have any effect on the development of the parthenogenetic merogone since the other red halves (from eggs fertilized, then centrifuged) do not develop and yet have this material also. The interesting feature of parthenogenetic merogony is that an egg will cleave and by successive cleavages form a blastula, without the presence of the mature ♀ nucleus or any substances from it, and without the ♂ nucleus. The protoplasm of a mature egg is necessarily a product of successive generations and it contains materials of the germinal vesicle from within its boundaries

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PLATE VI

Development of Whole Eggs Centrifuged After Fertilization

PHOTOGRAPH 59. Normal 2-cell stage (upper). Egg inside fertilization membrane, in which only white half has cleaved (lower left). Same without fertilization membrane (right). Centrifuged 45 minutes after fertilization. Photographed 2 hours after fertilization.

PHOTOGRAPH 60. Normal micromere stage. Centrifuged just before cleavage. Photographed 2½ hours after fertilization.

PHOTOGRAPH 61. Late cleavage stage, white and red portions distinct. Centrifuged 3 minutes after fertilization. Photographed 4 hours later.

PHOTOGRAPH 62. Blastula soon before hatching. Centrifuged 40 minutes after fertilization. Photographed 6 hours after fertilization.

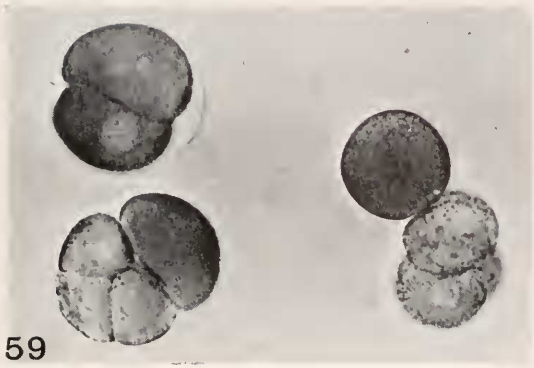
PHOTOGRAPH 63. Whole egg inside fertilization membrane, white portion only developed. Centrifuged just before cleavage. Photographed 6 hours after fertilization.

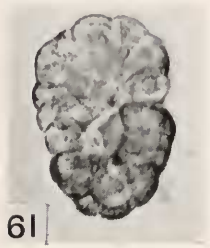
PHOTOGRAPH 64. Both parts develop with nuclei from original diploid nucleus. Centrifuged just before cleavage. Photographed 8 hours after fertilization.

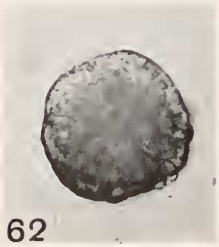
PHOTOGRAPH 65. Similar egg; two nucleate parts about to separate, forming twins. Centrifuged 30 minutes after fertilization. Photographed 9 hours later.

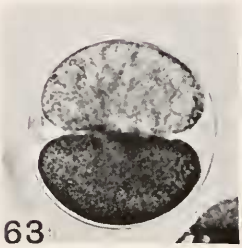
PHOTOGRAPH 66. Similar pair, 3 days old. Larger one is a white blastula, smaller a red gastrula. Centrifuged 40 minutes after fertilization.

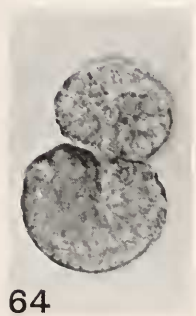
PHOTOGRAPHS 67, 68. Two white twins and a red blastula, all with nuclei from original diploid nucleus, forming triplets. Centrifuged soon before cleavage. Photographed 8 hours later.

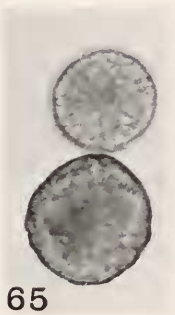


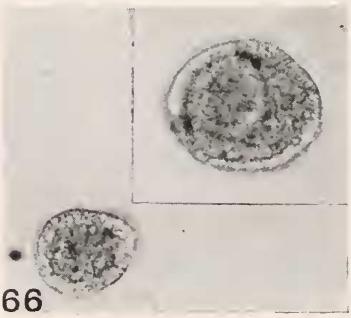












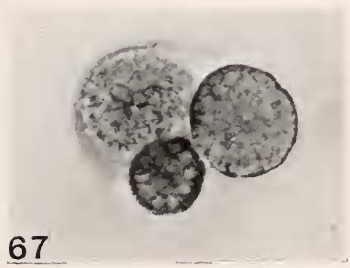


PLATE VI

as well as nutrient materials from without. Certainly the chromosomes and genes which are generally believed to determine its own fate are *not* present in a parthenogenetic merogone.

Since the non-nucleate half-eggs obtained after fertilization behave so differently from those obtained before fertilization and subsequently activated, it is obvious that a change has taken place in the protoplasm on fertilization which changes its developmental potencies as well as its physical and chemical characteristics such as permeability, viscosity and oxygen consumption. This change must take place very rapidly since the non-nucleate halves will not develop even if obtained as quickly as possible after fertilization nor can they be re-activated. The change in rate of stratification of the fertilized and unfertilized eggs also takes place immediately as well as the change in the rate and method of breaking (1933).

In comparison with the amoeboid activity of the red halves of the *Arbacia* egg, obtained after fertilization, it is of interest to refer to the classical work of E. B. Wilson (1904) on *Dentalium*. He found that the non-nucleate portion, obtained by cutting a fertilized egg, and containing the polar lobe, would go through rhythmic phases simultaneously with the cleavage of the nucleate portion and form a polar lobe just as though it were still a part of the complete egg, and even appeared as though it divided into two.

Development of Whole Eggs Centrifuged after Fertilization

Among the eggs broken apart by centrifugal force, there are always, with the forces used, some eggs which have not broken apart, both with and without fertilization membranes. These may develop quite normally like the uncentrifuged egg, even giving off micromeres (Photographs 59, upper egg; 60). Perfectly normal blastulae and plutei are formed. In some cases the stratification remains during cleavage and the egg may still remain elongate if the fertilization membrane has been removed (Photographs 61, 62), resembling the elongate eggs fertilized after centrifugation. In some cases, even within the fertilization membrane, the two parts, the light and the pigmented, may develop independently, and it frequently happens that the white portion cleaves and the red portion does not (Photographs 59, lower left and 63). If without the membrane, the two portions may be only partially separated and start to develop as a whole, both parts being nucleate (Photograph 64). These parts may become free-swimming blastulae and later separate and give a pair of twins, which are different in color, but with the same nuclear make-up (Photograph 65). Several of these pairs were isolated and in all cases after three days, the white twin was a blastula and the

red twin had invaginated (Photograph 66). It would be of interest to know whether these red halves with diploid nuclei would develop better than the fertilized merogones with haploid nuclei which are so difficult to raise. Together with these "twins" occur also "triplets," consisting of a red blastula, and two white blastulae which have apparently developed from the upper portions of the first two blastomeres; these three blastulae are, of course, all nucleate (Photographs 67, 68). My departure from Woods Hole prevented further investigation of these twins and triplets.

Summary

1. Fertilized eggs of *Arbacia* may be broken by centrifugal force into white and red halves similar to the unfertilized egg; the nucleus is in the white half.

2. The white half may develop quite normally through the blastula stage; no normal plutei have been obtained. The first two blastomeres may develop independently forming white twins. Amoeboid forms and loose masses of cells also result from the white half.

3. The red half does not cleave or develop. It may become amoeboid or notched, or form asters, thus indicating activation.

4. It makes little difference at what stage the eggs are centrifuged.

5. The red half cannot be refertilized or activated artificially.

6. Lack of development of the red halves obtained after fertilization is in striking contrast to the development of the red halves obtained before fertilization and subsequently activated artificially (parthenogenetic merogones).

7. Whole eggs may develop normally after centrifuging. They may separate later into 2 or 3 parts, forming nucleate red and white twins or triplets.

LITERATURE CITED

HARVEY, E. B., 1932. The development of half and quarter eggs of Arbacia punctulata and of strongly centrifuged whole eggs. *Biol. Bull.*, **62**: 155–167.

HARVEY, E. B., 1933. Effects of centrifugal force on fertilized eggs of Arbacia punctulata as observed with the centrifuge-microscope. *Biol. Bull.*, **65**: 389–396.

HARVEY, E. B., 1934. Effects of centrifugal force on the ectoplasmic layer and nuclei of fertilized sea urchin eggs. *Biol. Bull.*, **66**: 228–245.

HARVEY, E. B., 1936. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.*, **71**: 101–121.

HARVEY, E. B., 1938. Parthenogenetic merogony or development without nuclei of the eggs of sea urchins from Naples. *Biol. Bull.*, **75**: 170–188.

HARVEY, E. B., 1940. A new method of producing twins, triplets and quadruplets in Arbacia punctulata, and their development. *Biol. Bull.*, **78**: 202–216.

WILSON, E. B., 1904. Experimental studies on germinal localization. I. The germ regions in the egg of Dentalium. *Jour. Exper. Zoöl.*, **1**: 1–72.